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Leishmania specific CD4 T cells release IFN-γ that limits parasite replication in patients with visceral leishmaniasis

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Background: An immunological feature of VL patients is the inability of PBMC to respond to leishmanial antigen. But unexpectedly, it was recently found that *Leishmania* specific IFN-γ, can readily be detected when a whole blood stimulation assay is used. We explored the conditions that permit whole blood cells to respond to antigen stimulation, and clarify the biological role of the IFN-γ.

Methods & Materials: Whole blood and PBMC from VL patients were used and different cytokines were measured by ELISA. To test the role of complements, antibodies or other proteins present in plasma on SLA induced IFN-γ production, we replaced the plasma with heat inactivated FBS. Magnetic beads and column based depletion method were used to find the cellular sources of SLA induced IFN-γ production and further validated by intracellular cytokine staining. We employed an ex-vivo assay using splenic aspirate (SA) cells of active VL cases to assess the effect of IFN-γ blockade on parasite growth and pathology. Viable amastigote count and cytokine levels in culture supernatants were used as a read-out for the assay.

Results: CD4+T cells are main source of leishmania specific IFN-γ production in VL patients. Complement, antibodies and RBC do not play any significant role in the IFN-γ responses. However, removal of CD15+or CD56+cells reduced IFNγ production, suggesting that neutrophils and NK cells or other cells expressing these markers, contribute to the response in Whole Blood. Blockade of IFN-γ, increases amastigote numbers in SA cultures which can be argued to support their role in promoting immunity in patients with active disease.

Conclusion: Most patients with VL have antigen specific cells capable of secreting IFN-γ both in blood and spleen. Endogenous IFN-γ plays role in limiting parasite growth and could be considered as a potential target in immune based therapy alone or as an adjunct to reduce the dose and length of treatment.

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Amine modified graphene mediated drug delivery of Amphotericin B for the treatment of visceral leishmaniasis

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Background: We evaluated a novel formulation of Amphotericin B (AmB) conjugated to amine-modified graphene (f-Gr) for safety and efficacy over conventional AmB.

Methods & Materials: The f-Gr was prepared in a gentle one step process of non covalent (amine) functionalization with the help of amino acid L-cysteine. Non-covalent functionalization of graphene with π-π interactions works as the binding force between graphene and the ligand. This amine functionalized graphene sheet is further conjugated to AmB. The conjugate (f-Gr-AmB) was characterized by FTIR, SEM, TEM and Raman spectroscopy that established successful attachment of AmB to f-Gr. Cytoxicity of amine modified graphene Amphotericin B (f-Gr-AmB) is assessed in vitro against J774A.1 macrophage cell lines and in vivo in Swiss mice. Antileishmanial activity of f-Gr-AmB is tested *in vivo* in hamsters and against J774A.1 macrophage cell lines *in vitro.*

Results: FTIR, SEM, TEM and Raman spectroscopy showed successful attachment of AmB to f-Gr. The f-Gr-AmB was found to exhibit lesser cytotoxity towards J774A.1 cells than AmB, and did not induce any hepatic or renal toxicity in Swiss albino mice. *In vitro* antileishmanial assay in J774A.1 showed significantly enhanced efficacy of f-Gr-AmB over AmB. Furthermore, Percentage Inhibition of amastigote replication in hamster model of VL was significantly higher in f-Gr-AmB treated group (87.8%) compared to AmB (70.4%).

Conclusion: These results suggest that f-Gr-AmB could be a safe and effective alternative to conventional AmB in the treatment of VL.

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